

Sea lice monitoring on Atlantic salmon farms in New Brunswick, Canada: Comparing audit and farm staff counts

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## ABSTRACT:

Sea lice audits were performed by the Atlantic Veterinary College on commercial aquaculture sites in New Brunswick, Canada in 2011. Although the primary objective was to verify that farms were reporting similar lice counts to third-party counts, more detailed comparisons were developed to identify when lice counts were more likely to differ between the audit team and farm employees. A total of 28 sea lice audits were conducted on 16 sites between June and December, 2011. During each audit, 10 cages were evaluated per site where possible, with ten fish per cage being evaluated by an audit technician and a further ten by a farm employee. Data analysis included descriptive statistics of lice counts by stage and limits of agreement plots. A random effects negative binomial model which accounted for clustering of cages within sites was applied to assess the effect of counter type and season on lice counts by stage. The results indicate that farms counts were generally in agreement with audit counts. However, when the average counts for chalimus and preadult (male and female) and adult male lice stages were high, farm counters were more likely to report a lower value. Higher lice counts were observed during fall compared to summer especially for the adult female stage. Finally, there was a significant clustering effect for site and cage, with most of the variation attributable to site.

Keywords: Sea lice, monitoring, audit, clustering

## Introduction

Sea lice are parasitic copepods which feed on the mucous and skin of their host. Their impact can range from inconsequential effects to severe skin damage leading to mortality (Costello 2006).

*Lepeophtheirus salmonis* is the most common sea lice species affecting Atlantic salmon (*Salmo salar* L.) on the eastern coast of Canada and is the principal cause of damage attributed to lice infestation (Westcott, Hammell & Burka 2004). Under intensive production systems, such as cage-based aquaculture, sea lice can have a direct impact on the productivity of fish farming, particularly as their prevalence or abundance increases (Pike & Wadsworth 1999). This is particularly important on the east coast of Canada as high densities of fish farms can lead to the more rapid spread of lice infestation (Jansen, Kristoffersen, Viljugreun, Jimenez, Aldrin & Stein, 2012). In 2006, regional estimates of the cost of sea lice infection ranged from 4-10% of production value (Costello 2009). Although the recent economic impact of sea lice parasitism has not been assessed formally in New Brunswick, it is generally accepted to be the most costly disease agent, particularly since 2009 when the effectiveness of emamectin benzoate was reduced (personal communication, Mike Beattie, NB Dept of Agriculture and Aquaculture). Globally, sea lice are the most damaging parasite to salmonid farming and are estimated to cost the world industry €305m a year (Costello 2009). Monitoring sea lice numbers on a regular basis is important in order to make decisions about when to treat fish. The need for, and the choice and timing of treatments, will depend on the number of lice, and the stages that are present on the fish. Monitoring the level and development of sea lice abundance in farms is an important factor in the successful management of this parasite (Jackson, Hassett, Deady & Leahy 2000; Westcott *et al.* 2004; Heuch, Bjørn, Finstad, Holst, Asplin & Nilsen 2005). This is particularly important

when resistance to some treatments reduces the therapeutic options, as is the situation in New Brunswick, Canada (Jones, Hammell, Dohoo & Revie 2012).

Various authors have proposed monitoring procedures (Treasurer & Pope 2000; Revie, Gettinby & Wallace 2005; Heuch, Gettinby & Revie 2011). The most common procedure for estimating abundance and prevalence is to obtain representative samples of fish from multiple cages and report the number of lice, by stage, on each fish (Treasurer & Pope 2000). By necessity, these samples are not random but constitute a convenience sample (Heuch *et al.* 2011) that is presumed to be random. Revie *et al.* (2005) have suggested that monitoring procedures should involve sampling of a large number of cages using a small number of fish from each, since there is usually greater variation in lice numbers between cages than within cages.

In April 2011, the Atlantic Veterinary College (AVC) commenced an auditing program of on-farm sea lice monitoring of farmed salmon in aquaculture sites in the Bay of Fundy, New Brunswick (NB), Canada. This programme was part of the integrated sea lice management programme (ISLMP) implemented by the New Brunswick Department of Agriculture, Aquaculture and Fisheries (NBDAAF). The goals of the programme were: 1) to validate farm reported sea lice counts by performing independent sea lice assessments and comparing audit results with data provided by individual companies, 2) to evaluate the precision of sea lice monitoring using farm-reported data, 3) to identify areas where training programmes are required, and 4) to achieve consistency in sea lice counts between individual farm counters so that comparisons across locations and time within the industry are reliable.

## **Materials and Methods**

### **Data collection**

Marine Atlantic salmon aquaculture sites in the Bay of Fundy, NB, Canada, were monitored as part of the ISLMP. The dataset consisted of sea lice counts that were conducted by farm staff and an audit team on 16 sites from June to December 2011, involving a total of 28 audits. During each audit, 20 fish per cage and 10 cages per site were sampled (when sufficient cages were available). Each audit technician and site worker counted 10 different fish, collected on the same day and time from the same cage following a standard “feed and dip” method (i.e. feed used as an attractant then fish obtained with a dip net) of fish selection (Treasurer & Pope 2000; Ersdal, Midtlyng & Jarp, 2001; McClure, Hammell, Dohoo, Nerette & Hawkins 2004). Fish were anaesthetized with TMS (tricaine methanesulfonate) at a dose of 50 - 100 mg/L. Lice species, number, and stages groups, categorised as: a) chalimus, b) preadult (male and female) and adult male (PAAM), and c) adult female (AF), were recorded independently by each counter, blinded to the records of the other counter.

### **Statistical analyses**

All analyses were conducted at the cage level. Descriptive statistics (mean, median, range, and percentiles) were computed for each lice stage (chalimus, PAAM, and AF) by counter (audit technicians vs. farm staff) over the study period. A histogram was used to illustrate the overall distribution of lice count for each stage. Limits of agreement plots (LoA, (Bland & Altman 1986)) were used to assess the agreement between lice count conducted by farm staff and by audit technicians. Finally, a random effects negative binomial model was used to assess the effect of counter type (audit technicians vs. farm staff) and season (summer vs. fall) on lice counts. This model was used to account for overdispersion in the data (mean greater than variance) and to account for clustering of cages within sites (Dohoo, Martin & Stryhn 2009). To obtain an integer number to be used in the statistical count model, the dependent variable was

derived by multiplying cage level mean counts by 10. Separate models were fitted for each lice stage. The Vuong statistic was used to assess the fit of a zero inflated versus a standard negative binomial model. All analyses were conducted using Stata version 12 (Stata Corp., College Station, TX).

## **Results**

### **Descriptive statistics**

Over the study period, a total of 3200 fish were sampled from 178 cages during 28 audits of 16 unique sites. Figure 1 shows that chalimus, PAAM and AF counts have strongly right skewed distributions at the cage level with the majority of counts being close to zero. Table 1 provides summary statistics of lice count by counter and lice stage at the cage level. In general, the mean count per cage was low and was less than one for chalimus and AF stages, regardless of the counter type, whereas for preadults and adult male the mean count per cage was less than two. In addition, approximately 35% of the chalimus and AF counts were reported as zeros compared to 17% for the PAAM stages. Finally, the overall variance was substantially greater than the mean especially for PAAM and AF stages.

### **Limits of agreement plots**

Figure 2 shows the LoA plots for different lice stages. For PAAM, all counts were located within the 95% LoA at low levels of lice count (less than three). As the magnitude of counts increase, farm staff counts tend to be lower than audit counts. Similar trends were observed for chalimus and AF stages, although differences in chalimus counts tended to occur when the mean count was greater than 1.5 lice. The graph also shows that most of the differences between the farm staff and audit counts fell approximately in the range of -2 and +2 for both chalimus and AF, whereas in case of PAAM, most of the counter differences fell between -3 and +2 lice.

## **Multivariable model**

In all lice stages, the overall variance was greater than the mean, hence a Poisson model was not considered appropriate since it assumes the mean and the variance to be approximately equal. Table 2 shows the results from the random effects negative binomial models for chalimus, PAAM, and AF stages. The models indicate a significant difference between the counts recorded by the audit technicians and the farm employees for chalimus and PAAM stages, with the audit technicians recording higher counts than farm employees. There were no significant differences between summer and fall counts for chalimus or PAAM stages, however fall counts were significantly higher for the AF stage. The alpha values (2.26-4.54) indicate that the negative binomial model fit the data better than the Poisson model. Due to the presence of zero counts (17-35% depending on lice stage), a zero-inflated negative binomial model was also fit to the data. However, the Vuong test indicated that a standard negative binomial model fit the data better than the zero-inflated model. Finally, there were significant clustering effects for site and cage. The intra-class correlation (ICC) was 0.46, 0.78, and 0.60 for chalimus, PAAM, and AF, respectively, indicating that most of the variation, for mobile stage counts in particular, was attributable to site-to-site variance.

## **Discussion**

Although considerable research effort has been expended on sea lice control over the past three decades, these ecto-parasites remain a persistent problem in most countries where Atlantic salmon are cultured. As part of the integrated pest management approach, regular monitoring of sea lice by farms is required to avoid fish being exposed to detrimental levels of infestation and to evaluate treatment efficacy ( Heuch *et al.* 2005; Revie, Gettinby, Grant & Reid 2002; Lees, Baillie, Gettinby & Revie 2008). In New Brunswick, sea lice counts are currently conducted on

each salmon farm site once per week between mid-April and December when the water temperatures are greater than 4 °C (max of 18.8 °C in this dataset) and sea lice develop most rapidly (Tucker, Sommerville & Wotten, 2001). Although sea lice are not as much of a concern at colder water temperatures, due to a decrease in their feeding activity, growth and reproductive potential, they are still capable of overwintering on salmon and as such require continued monitoring (Boxaspen, 2006). Therefore, monitoring is attempted by most sites at least once per month when water temperatures are less than 4°C (i.e. between January and mid-April). It is essential that lice counts have repeatability across sites so that treatment threshold identification and trend comparisons are referring to similar occurrences. This becomes more important as lice burdens increase and site managers must reliably estimate the reduction in lice stages that are attributed to any treatments administered. Although, the overall mean lice counts per cage were low (less than one for chalimus and AF, and less than two for PAAM), some differences were observed between audit technician and farm staff counts. These differences were illustrated in the LoA plots which show good agreement between farm staff and audit technicians for most of the evaluated cages, with only 4, 7, and 5% of the cages outside the LoA for chalimus, PAAM, and AF, respectively. Most of the points outside the LoA occurred at higher magnitude of counts and farm staff counts tended to be lower than audit counts. Although an increase in variation is expected at higher magnitude counts, the difference between the two counter types should be distributed evenly on both sides of the 95% LoA interval. A clinically acceptable threshold of difference could be specified *a priori* for each lice stage as an acceptable LoA; these can then be used to identify sites where training may be required.

The reported lice counts were not statistically independent because cages were clustered within sites, therefore differences in lice counts between audit technicians and farm staff were tested



using random effects negative binomial models. A highly significant difference between counters was observed for chalimus and PAAM stages, with the audit technicians having a positive coefficient indicating that audit technicians generally reported higher counts than farm staff. On the other hand, there was no significant difference between counter types for the AF stage. These findings may be related to the fact that the AF stage is larger in size and therefore can be easily distinguished by all counters, in comparison to larval or pre-adult stages which are smaller and can be more easily overlooked by inexperienced counters. In contrast, Heuch *et al.* (2009) did not find a significant effect of counter type (trained teams vs. farm staff) in chalimus or PAAM counts. However, in our study audits and farm staff comparisons were made at the cage level, whereas counter comparison by Heuch *et al.* (2009) were made at the site level. Another study that evaluated the effect of counter type was implemented by the Ministry of Agriculture and Lands, BC, Canada (Saksida, Constantine, Karreman & Donald 2007a), and reported no significant differences between counters in the majority (28/32) of the comparisons of lice by stage using mean values averaged over all farms in a given quarter, with farm staff estimates being higher than audit estimates in the majority of cases where disagreement occurred. Direct comparison between the current study and the other two studies cannot be made due to variations in the level at which counts were reported (i.e. cage vs. site vs. all sites in a quarter), however aggregating data at the site level is expected to obscure some variations between counters that may occur at the cage level. More detailed comparisons are possible when assessments are made at the cage level.

A potential bias may have occurred due to the use of feed and dip method for sampling of fish. Healthier fish, with lower lice count, may be more attracted to the food and more likely to be sampled, however this type of bias should be equally distributed between counter type and if this

bias has any effect on the reported association between counter type and lice count, it will be toward the null (i.e. reducing the strength of association between counter type and lice count). Seasonal variations in lice counts were compared for the two seasons (summer vs. fall) during which data were collected. Previous studies reported that the distribution of *L. salmonis* on farmed Atlantic salmon vary both spatially and temporally depending on a range of factors including seasonality, site location and treatment intervention (Revie, Gettinby, Treasurer & Wallace 2003; Jansen *et al.* 2012). In this study, higher counts were observed during fall compared to summer for all lice stages, although this effect was only significant for the AF stage. Variation in lice counts is expected in different seasons due to fluctuation in water temperature, with increases in louse abundance at higher water temperature due to shorter generation times (Costello 2006). Various studies (Revie *et al.* 2003; Saksida, Karreman, Constantine & Donald 2007b) found no significant effect of water temperature on abundance level of *L. salmonis*. The lack of effect may reflect the fact that seasonal variation in lice counts is a function of changes in water temperature interacting with changes in management factors through different seasons. Similar seasonal trends were observed by Saksida *et al.* (2007b) in BC, with higher lice abundance occurring in fall and winter, whereas the lowest abundance was observed during the summer. Seasonal variations have been accommodated in monitoring programs by requiring more frequent reporting during periods with higher abundance.

Finally, significant clustering effects were observed for site and cage, with ICC values for mobile lice that ranged from 0.60 (AF) to 0.78 (PAAM). These values reflect that most of the variations in mobile lice stages were attributed to between-site variations, whereas variation (ICC of 0.46) in chalimus counts could be roughly distributed equally to the site and cage levels. Previous studies by (Revie *et al.* 2005; Heuch *et al.* 2011; Revie, Hollinger, Gettinby, Lees &

Heuch 2007) reported that significant levels of clustering of lice counts can occur within and between cages in salmon farms. The recommendation to sample large numbers of cages with smaller number of fish was based on ICC values which indicated larger variation between than within cages. In this study, we looked at clustering of cages within sites, whereas other studies looked at clustering of fish within cages without taking into account between-site variations. It is recognised that the similarity between cages may be related to lower overall levels of abundance observed in this study. However, the ICC values found for mobile stages in our study were higher than the ICC value for the chalimus stage which agrees with previous observations (Revie *et al.* 2005; Revie *et al.* 2007). Further analysis that considers all levels of clustering (i.e. fish within cages within sites) will be required to obtain a more complete picture about the distribution of variance at each level of clustering.

In conclusion, farms counts were generally in agreement with audit counts. However, when the average counts for chalimus and PAAM lice stages were high, the farm counters were more likely to report a lower value, a trend which could be attributed to inexperienced counters at some sites. Seasonal variation should be considered when comparing counts from different sites.

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Table 1: Descriptive statistics of chalimus, preadult (male and female) and adult male, and adult female count by counter at the cage level (n=178)

Stage	Counter	Range	25%	Median	75%	Mean	Var.
Chalimus	Audit technician	0-6.28	0	0.20	0.60	0.56	0.92
	Farm staff	0-3.40	0	0.10	0.40	0.33	0.30
Preadult (male and female) and adult male	Audit technician	0-22	0.10	0.60	2.60	2.11	13.38
	Farm staff	0-16.40	0	0.40	1.80	1.49	7.54
Adult female	Audit technician	0-19	0	0.10	0.71	0.98	7.09
	Farm staff	0-13.8	0	0.10	0.67	0.83	3.97

Table 2: Random effects negative binomial models comparing cage level sea lice counts performed by farm staff and audit technicians for chalimus, preadult (male and female) and adult male, and adult female stages.

Covariates	Chalimus		Preadult (male and female) and adult male		Adult female	
	Estimate (SE)	P	Estimate (SE)	P	Estimate (SE)	P
Counter (ref=Farm staff)						
Audit technician	0.48 (0.11)	0.000	0.31 (0.06)	0.000	0.04 (0.06)	0.595
Season (ref=Summer)						
Fall	0.65 (0.46)	0.157	1.07 (0.60)	0.060	1.61 (0.74)	0.030
Intercept	0.92 (0.40)	0.021	2.16 (0.40)	0.000	1.24 (0.53)	0.019
alpha	2.26 (0.47)		2.38 (0.52)		4.54 (1.02)	

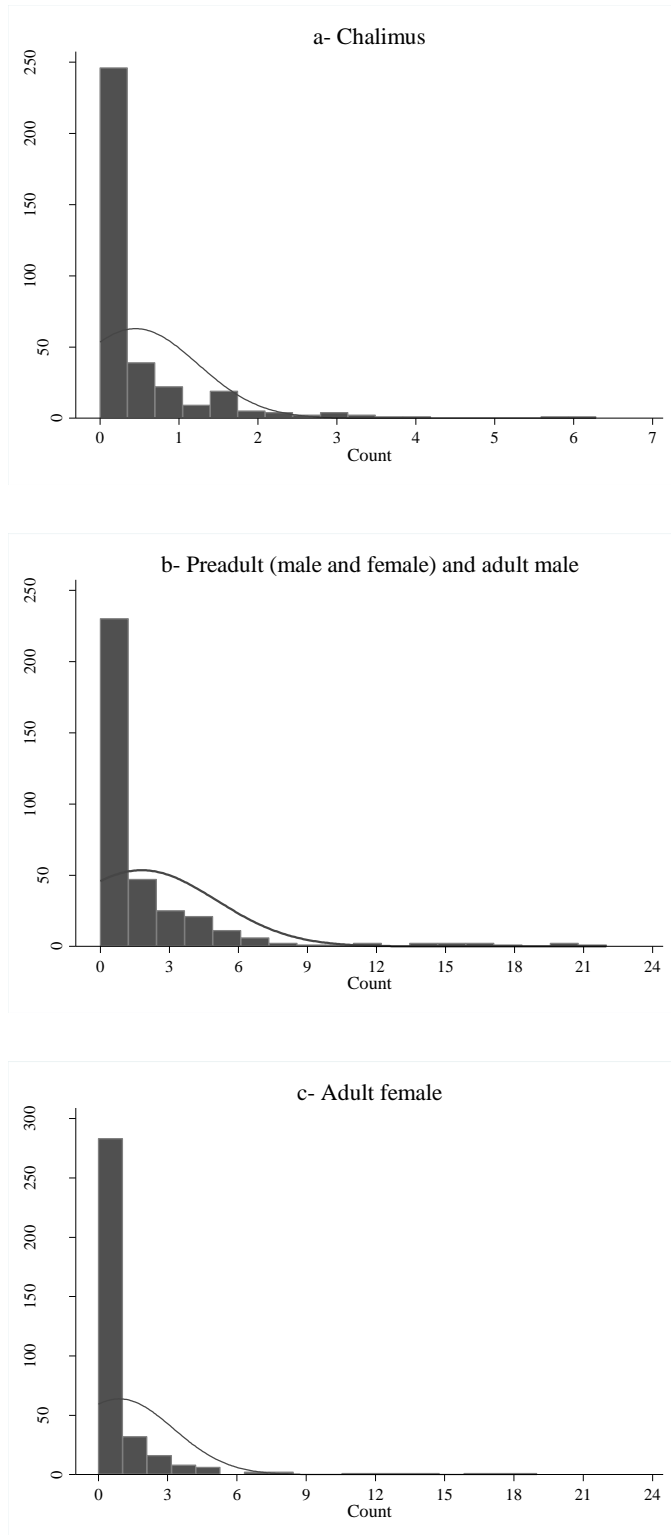


Figure 1. Cage level distribution of the overall a) chalimus, b) preadult (male and female) and adult male, and c) adult female counts based on 178 cages from 16 unique sites in NB, Canada, over the period from June to December, 2011.



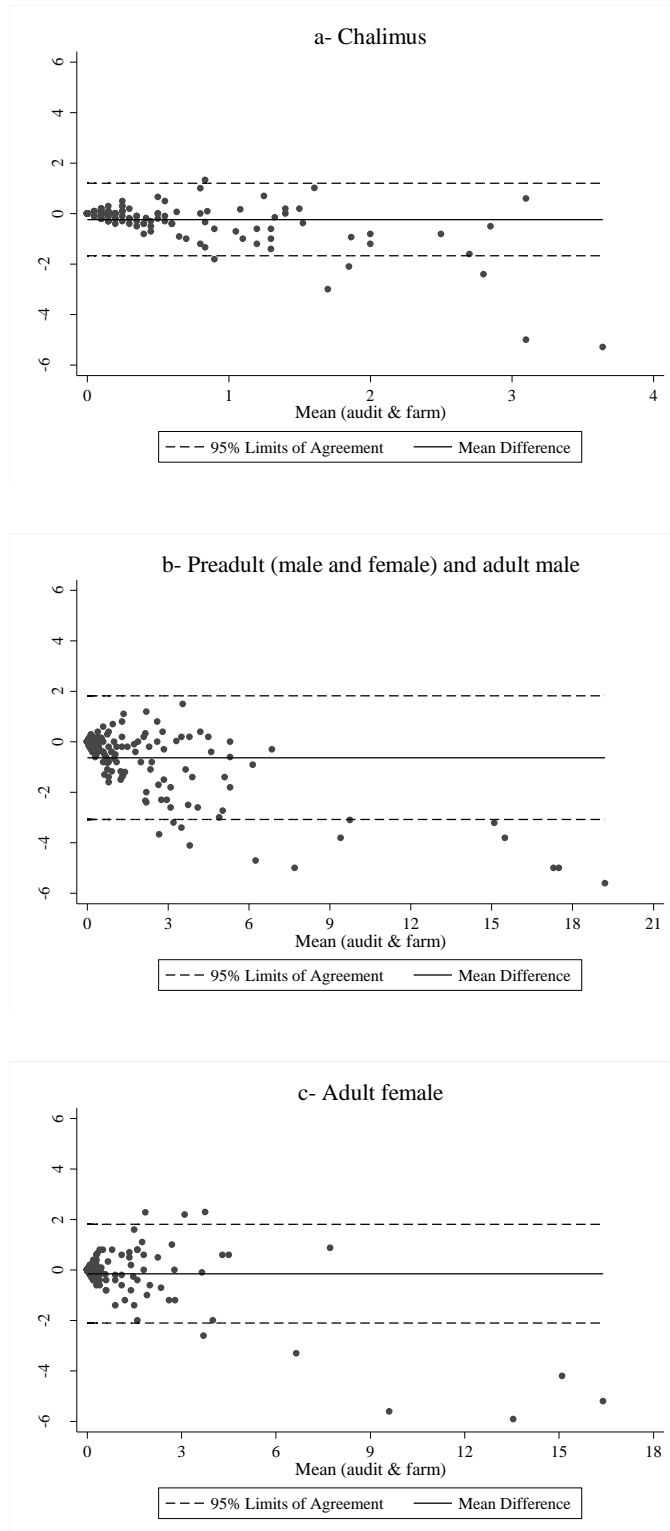


Figure 2. Bland–Altman plots showing the difference against the average of farm staff and audit technician counts for a) chalimus, b) preadult (male and female) and adult male, and c) adult female.